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Alteration of CREB phosphorylation and spatial memory deficits in aged 129T2/Sv mice

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Abstract

Phosphorylation of cAMP-response element binding protein (CREB) is required for hippocampus-dependent long-term memory formation. The present study was designed to determine whether spatial memory deficits in aged mice were associated with alteration of hippocampal CREB phosphorylation. We examined the temporal pattern of CREB activation in 5–6 and 23–24-month-old 129T2/Sv mice trained on a spatial reference memory task in the water maze. Phosphorylated CREB (pCREB), total CREB (t-CREB) and c-Fos immunoreactivity (ir) were measured at four time points after the end of training. In young mice, pCREB-ir was significantly increased 15 and 60 min after training in the CA1 region and dentate gyrus. In aged mice sacrificed 15 min after training, pCREB-ir in these structures was reduced whereas t-CREB-ir remained unchanged compared to respective young-adults. An age-related reduction of c-Fos-ir also occurred selectively in hippocampal CA1 region. Since reduced pCREB-ir in CA1 from the 15 min-aged group strongly correlated with individual learning performance, we suggest that altered CREB phosphorylation in CA1 may account for spatial memory impairments during normal aging.

Keywords: Aging; Memory; CREB; Fos; Hippocampus; Immunohistochemistry; Water maze

1. Introduction

Memory capabilities that depend on the hippocampus functional integrity are particularly vulnerable to the aging process (Foster, 1999). Studies in multiple species indicate that normal aging is associated with functional deficits in a variety of hippocampus-dependent memory tasks (Bach et al., 1999; Barnes, 1979; Brightwell et al., 2004; Burke and Barnes, 2006; Gallagher and Rapp, 1997; Head et al., 1995; Mons et al., 2004; Monti et al., 2005; Rapp et al., 1997; Rosenzweig and Barnes, 2003; Touzani et al., 2003). In both humans and rodents, aging is associated with declines in hippocampal-dependent long-term memory (LTM) consolidation and alterations in the maintenance of hippocampal long-term potentiation (LTP) (Barnes and McNaughton, 1985; Geinisman et al., 1995; Landfield et al., 1978; Lynch, 1998). Among the multiple factors that are strategically positioned to contribute to memory deficits during normal aging lies the transcription factor CREB (cAMP response element binding protein). Ample evidence from a variety of species indicates that CREB is critical for consolidation of LTM (for reviews, Izquierdo et al., 2002; Lamprecht, 1999; Silva et al., 1998) and long-lasting LTP (Bourtchuladze et al., 1994; Impey et al., 1996; Miyamoto, 2006; Mizuno et al., 2002; Schulz et al., 1999). Indeed, CREB activation through its phosphorylation on Serine-133 (pCREB) controls the induction of regulatory immediate-early genes (IEG) whose products, in turn, induce the transcription of late downstream genes, and activate direct "effector" proteins, such as structural proteins, signaling enzymes or growth factors, that are essential for LTM (Lanahan and Worley, 1998; Lonze and Ginty, 2002). A specific and temporally controlled inhibition of CREB function disrupts the performance in various

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forms of hippocampus (Florian et al., 2006; Guzowski and McGaugh, 1997), amygdalar (Josselyn et al., 2004) or striatal (Pittenger et al., 2006) dependent memories. Conversely, increasing expression of CREB in the basolateral amygdala specifically enhances LTM for fear conditioning (Josselyn et al., 2001). Furthermore, mutant mice expressing a dominant negative form of CREB exhibit deficits in different forms of LTP and memory (Huang et al., 2004; Pittenger et al., 2002).

With respect to aging, recent studies have shown close relationship between age-related cognitive impairment and dysregulation of CREB activation due in particular, to the lack of increased phosphorylation upon learning in aged rats (Asanuma et al., 1996; Brightwell et al., 2004; Foster et al., 2001; Hattiangady et al., 2005; Kudo et al., 2005; Monti et al., 2005). However, the age-related changes in the CREB phosphorylation within the rat hippocampus after learning are controversial, likely due to differences in the age, strain and sex of animals and the methodology used for the different analyses. Recently, Mouravlev et al. (2006) reported that elevation of hippocampal CREB levels achieved by somatic gene transfer can prevent memory loss in aging rats subjected to different Barnes behavioral tasks. In contrast, intra-hippocampal administration of drugs that enhance the cAMP-protein kinase A (PKA) signaling pathway can reverse the age-related defects in both spatial memory and late LTP in aged C57Bl/6 mice (Bach et al., 1999) while activation of the same signaling cascade impairs prefrontal cortical functions in aged rats and monkeys with working memory deficits (Ramos et al., 2003). Altogether, these studies indicate that changes in the phosphorylation state of CREB contribute to memory deficits during normal aging. However, there is little information concerning the possible effects of aging on the amplitude and the temporal profile of CREB phosphorylation. Indeed, previous studies have shown that the duration of cAMP- and calcium (Ca^{2+})-mediated CREB phosphorylation is a major determinant of cAMP response element (CRE)-mediated gene expression, such as *c-fos* (Baker et al., 2004; Johannessen et al., 2004; Liu and Graybiel, 1996).

To establish a more definitive link between brain aging, altered CREB activity and spatial memory impairments, we analyzed by immunohistochemistry the regional and temporal patterns of phosphorylated CREB (pCREB) within the different hippocampal subregions of young-adult and aged 129T2/Sv mice subjected to three days of a reference learning task in the Morris water maze. Previous studies have shown that this task is particularly sensitive to age-related deficits in hippocampal-dependent memory (Frick et al., 1995; Lindner, 1997; Wolff et al., 2002). Levels of pCREB, total CREB (t-CREB) and c-Fos protein levels were measured in young-adult and aged mice sacrificed 15, 60, 90 min and 8 h after the last training trial. Our results indicate that altered CREB phosphorylation, particularly in CA1 region, was strongly associated with spatial memory deficits in aged 129T2/Sv mice.

2. Materials and methods

2.1. Behavioral procedures

2.1.1. Animals

The subjects were male 129T2/Sv mice (Wyss et al., 2000). On receipt from the breeding colony (Laboratoire de Transgénèse, Université de Bordeaux 2), they were housed individually in standard transparent laboratory cages (26 cm × 12 cm × 14 cm) in a temperature-controlled (22 ± 1 °C) colony room, near the experimental room. They were provided with food and water ad libitum, and maintained on a 12-h light:12-h dark artificial cycle (lights on at 7:00 h). At the beginning of the experiments the subjects were 5–6-month-old (young-adult, $n = 37$) and 23–24-month-old (aged, $n = 33$). Young-adult and aged mice were subjected to spatial learning in the Morris water maze, whereas caged naive mice (young: $n = 8$; aged: $n = 3$) served as controls. All mice were tested during the light phase between 10:00 and 17:00 h. All experimental procedures were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.1.2. Apparatus

The apparatus was a white circular tank (140 cm in diameter, 40 cm in height). It was located in a room with various distal cues and uniformly illuminated by a halogen lamp. The tank was filled with water (30 cm depth) maintained at 22 °C, and was rendered opaque by the addition of a non-toxic white paint (Pebeo, Gemenos, France). Located inside the pool was a removable circular (13 cm in diameter) Plexiglas hidden platform positioned such that its top surface was 0.5 cm below the surface of the water. It was placed in the middle of one virtual quadrant (north) of the pool. Data were collected using a video-camera fixed to the ceiling of the room and connected to a video-tracking system (Videotrack, Viewpoint, Lyon, France) located in an adjacent room.

2.1.3. Pretraining

During the day preceding learning proper, each mouse received a pretraining session in order to acquire the procedural aspects of the task and for controlling motor and visual capacities (Malleret et al., 1999).

2.1.4. Learning

Each mouse was submitted to a daily session comprising four trials over 3 successive days (Days 1–3). Each trial consisted of releasing the mouse into the water facing the outer edge of the pool at one of the virtual quadrants (except the quadrant where the platform was located), and allowing it to escape to the platform. A trial terminated when the animal reached the platform where it was allowed to remain for 15 s. The few mice, which, on the first day, failed to find the platform before 90 s elapsed, were invited to follow the finger of the experimenter which indicated the location of the platform, where they were allowed to remain for 15 s.